ABSTRACT

Aim: The purpose of this study was to evaluate the anti-diabetic effect of ethanolic extracts of *Petersianthus macrocarpus* leaf and its phytochemical analysis using different solvents.

Place and Duration of Study: The study was carried out between March and September in 2018 in the Department of Anatomy, Madonna University, Elele, Rivers State, Nigeria.

Materials and Methods: Twenty five Wistar rats, weighing between 200-225g were divided into five groups of five rats. Group A (Control) while Groups B, C, D and E were induced with diabetes using streptozotocin firstly 35mg/kg b.w; and 2 weeks later 25mg/kg b.w. Group B (Diabetic control), Group C received 0.5mg/kg b.w of Glibenclamide. Groups D and E received daily 50 and 100 mg/kg b.w of ethanolic leaf extract of *P. macrocarpus* orally for two weeks. The fasting blood glucose levels were determined weekly for two weeks. At the end of the experiment, the animals were sacrificed and the pancreas was removed for histological procedures.

Results: The body weights increased significantly (P<0.05) in 100 mg/kg b.w group when
compared to the diabetic control rats weight at the end of the experiment. Also there was a significantly decreased (P<0.05) in blood glucose levels in *P. macrocarpus* (100mg/kg b.w). The histological section of the pancreas of diabetic control showed eosiinophilic material in the islet, shrinkage of the islet of Langerhans while the group treated with 100 mg/kg of extract showed granulated and well prominent pancreatic islet of Langerhans. Phytochemical screening showed methanolic extract of *P. macrocarpus* leaf having alkaloid, saponin, tannin, phenol, flavonoid, cardiac glycoside, steroids, terpenoids, anthocyanin and anthraquinone. However, cardiac glycosides and steroids were absent in ethanolic extract. Cardiac glycoside and terpenoids were also absent in hexane and acetone extract, while phenol, cardiac glycosides, steroids and anthocyanin were absent in the water extract.

**Conclusion:** Ethanolic leaf extract of *P. macrocarpus* ameliorate streptozotocin-induced diabetes in Wistar rats.

**Keywords:** Petersianthus macrocarpus; diabetes; pancreas; STZ; phytochemical evaluation.

1. INTRODUCTION

It has been reported that herbal remedies may have recognizable therapeutic effects [1]. However, some species of plants have been reported to have hypoglycemic effects; hence, attention has been given to find biologically active compounds from various medicinal plants as a source of natural anti diabetic drugs [2,3,4]. However, only few of these plant have been reported [5]; *Chrysophyllum albium* [6], *Paracentrotus lividus* [7], *Vernonia amygdalina* and *Gongronema latifolium* [8], *Annona muricata* [9].

*Petersianthus macrocarpus* belongs to the family Lecythidaeae. It is widely used in folkloric medicine in the treatment of various ailments such as pains, fever associated with malaria and also as an anti-cancer agent [10]. It is also used to treat infections and gastrointestinal disorders [11]. Scientific studies have reported its antiproliferative activity in the leaf [12]; while the stem barks have antiplasmodial, analgesic, antioxidant, antinociceptive activities [13,14,15]. The fruit have been reported to have antidiabetic effect [16].

Diabetes mellitus is a metabolic condition in which the pancreas does not secrete sufficient insulin, or the body does not respond properly to insulin [17]. This causes glucose to accumulate in the blood, often leading to various metabolic complications [18]. Experimental diabetes can be induced by using streptozotocin which selectively destroy the pancreatic beta-cells through reactive oxygen species (ROS) production. Streptozotocin have been known to induce both type 1 and type 2 diabetes mellitus [19]. The islets which represent the endocrine part of the pancreas contain the alpha (A) cells, the beta (B) cells mainly, delta (D) cell, and C-cell. The Beta-cells which produce insulin are numerous forming about 70% of the islet cells and occupy the interior of the islet. Therefore, absolute or relative lack of insulin results in diabetes [20,21].

To the best of our knowledge, no work has been done or published on the anti-diabetic effects of the leaves of *Petersianthus macrocarpus*. Hence, in this study, we investigated antidiabetic potential of ethanolic extract of *P. macrocarpus* leaf and its phytochemical contents.

2. MATERIALS AND METHODS

A total of twenty-five Wistar rats of both sexes, weighing 200 to 225 g were obtained from the animal house of University of Nigeria,Nsukka. The animals were housed in the Department of Anatomy, Madonna University, Elele, Rivers State. The animals were left to acclimatize for 7 days in a well ventilated cages before the commencement of the experiment. The animals were also fed with standard diet and water ad libitum.

2.1 Collection of Plant Material

The leaf of *Petersianthus macrocarpus* were collected from Chileke farm Umunzeri Aguneze Ahiara Mbaise, Imo State, Nigeria. It was duly authenticated in the Department of Botany of the University of Nigeria, Nsukka. The rules for animal experiments was complied with according to the guidelines provided by the Guide for the Care and Use of Laboratory Animals [22].

2.2 Preparation of Leaf Extracts of *P. macrocarpus*

The leaves of *P. macrocarpus* were collected and air-dried for 21 days. They were then pulverized by a conventional milling machine.
A total of 1 kg of plant material was obtained. 1000 g of powdered material was macerated in 70% ethanol at room temperature for 48 h. The extract was filtered using a filter paper (Whatman No. 1). The extract is recovered from filtration by evaporation using rotary evaporator and concentrated further with water bath set at about 40ºC to yield 40.56 g of greenish brown substances. The semi-solid extracts obtained were stored in the refrigerator at 4ºC for further use.

2.3 Phytochemical Screening

Dried leaves of *P. macrocarpus* were crushed into powdered. The leaf was extracted using different solvents and the extract was screened for the presence or absence of secondary metabolites using phytochemical screening procedures described by [23,24,25].

2.4 Acute Toxicity Studies

Acute oral toxicity study was perform using Wistar rats according to the Organization for Economic Co-operation and Development (OECD) guideline 425 [26]. A total of ten male Wistar rats were fasted overnight and divided into two groups with five rats in each. The first group was the control and received distilled water while the second group was administered ethanol extract of *P. macrocarpus* at a limit test dosage of 5000 mg/kg body weight. All doses were administered orally and the animals were observed at 0 min, 30 min, 1 h, 2 h, 4 h, 6 h; every day for 14 days.

2.5 Induction of Experimental Diabetes Mellitus

The animals were fasted overnight with free access to water prior to the induction of diabetes. Diabetes was induced by single intraperitoneal injection of freshly prepared solution of streptozotocin (35mg/kg body weight) in a 0.1 M citrate buffer (pH 4.5), which was obtained from Sigma St Louis, M.O., USA. After 72 hours of streptozotocin injection, fasting blood glucose level was determined. Two weeks later, the animals were given a second intraperitoneal injection of STZ (25mg/kg b.w.). Fasting blood glucose level was also determined. Animals with fasting blood glucose level above 200 mg/dl were considered to be diabetic.

2.6 Experimental Design

After the induction of diabetes mellitus, the animals were divided into five groups of five rats. All animals were fasted overnight before treatment. Groups B, C, D and E were induced with diabetes except Group A that serves as the control. Treatment with the leaf extract and glibenclamide lasted for 14 days. The rats were divided as follows;

- **Group A**: Control rats (received 5 ml normal saline/kg b. w.).
- **Group B**: Diabetic rats (received 5 ml normal saline/kg b. w.).
- **Group C**: Diabetic rats treated with glibenclamide (0.5mg/kg b. w.).
- **Group D**: Diabetic rats treated with 50 mg/Kg b.w. of ethanolic extract of *P. macrocarpus*.
- **Group E**: Diabetic rats treated with 100 mg/Kg b.w of ethanolic extract of *P. macrocarpus*.

2.7 Determination of Blood Glucose Levels

Fasting blood glucose levels was obtained by puncturing the tail vein of the rats, using the Accuchek glucometer (Avina Nano, Roche diagnostic, Penzberg Germany) and results were reported as mg/dl. However, fasting blood glucose of each group was determined weekly throughout the experiment.

2.8 Termination of the Experiment

At the end of the 14th day of experiment after an overnight fast, the rats from each group were euthanized with diethyl ether. The pancreas of the various groups were harvested and placed in separate bottles which contained 10% formal saline. Tissue sections were cut and stained with haematoxylin and eosin stains.

2.9 Statistical Analysis

Statistical comparisons were analyzed using graphpad prism version 5.02. Data were presented as mean ± SD using one way analysis of variance (ANOVA) followed by Bonferroni post-hoc test. At P values < 0.05 is considered significant.
3. RESULTS

3.1 Acute Toxicity Study

Oral administration of the doses of the *P. macrocarpus* leaf extract to the rats did not show any significant changes in breathing, behavioral patterns, itching, sensory nervous system responses and all the rats survived throughout the experiment. Hence, the LD50 of the extract is said to be greater than 2000 mg/kg, which is reasonably safe.

3.2 Phytochemical Analysis of the Leaf of *P. macrocarpus*

The phytochemicals found in methanol extract of *P. macrocarpus* leaf were; alkaloid, saponin, tannin, phenol, flavonoid, cardiac glycoside, steroids, terpenoids, anthocyanin and anthraquinone. However, cardiac glycosides and steroids were absent in ethanol extract. Cardiac glycoside and terpenoids were absent in hexane and acetone extract while phenol, cardiac glycosides, steroids and anthocyanin were absent in water extract (Tables 1 and 2).

3.3 Body Weight

It was observed that there is a gradual decrease in the body weight of animals in diabetic control group. The animals treated with *P. macrocarpus* and glibenclamide showed a gradual increase in their body weight after 14 days of treatment. Among two test doses, *P. macrocarpus* at 100 mg/kg showed significant (p<0.05) recovery on body weight of the animals when compare to that of the diabetic control. The results are shown in Table 3.

3.4 Biochemical Changes

*P. macrocarpus* and glibenclamide lowered the blood glucose level gradually and this continued up to the 14th day (P < 0.05). However, *P. macrocarpus* at 100 mg/kg showed significant (P < 0.05) reduction in blood glucose. The results are shown in Table 4.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Parameter</th>
<th>Water</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Hexane</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Saponin</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Tannin</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Phenol</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Cardiac glycosides</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>7</td>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Anthocyanin</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Antraquinone</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: +++ Abundantly present, ++ moderately present, + slightly present, - Not detected

<table>
<thead>
<tr>
<th>S/N</th>
<th>Parameter</th>
<th>Water</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Hexane</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
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<td>2.94</td>
<td>1.93</td>
<td>1.42</td>
<td>1.29</td>
</tr>
<tr>
<td>2</td>
<td>Saponin</td>
<td>3.42</td>
<td>3.76</td>
<td>3.18</td>
<td>2.04</td>
<td>2.11</td>
</tr>
<tr>
<td>3</td>
<td>Tannin</td>
<td>3.16</td>
<td>3.55</td>
<td>3.72</td>
<td>1.88</td>
<td>1.50</td>
</tr>
<tr>
<td>4</td>
<td>Phenol</td>
<td>-</td>
<td>1.43</td>
<td>0.41</td>
<td>0.11</td>
<td>0.16</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoid</td>
<td>1.08</td>
<td>1.27</td>
<td>1.30</td>
<td>1.04</td>
<td>1.08</td>
</tr>
<tr>
<td>6</td>
<td>Cardiac glycosides</td>
<td>-</td>
<td>1.03</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Steroids</td>
<td>-</td>
<td>0.89</td>
<td>-</td>
<td>0.65</td>
<td>0.43</td>
</tr>
<tr>
<td>8</td>
<td>Terpenoids</td>
<td>1.04</td>
<td>1.31</td>
<td>1.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Anthocyanin</td>
<td>-</td>
<td>1.25</td>
<td>1.13</td>
<td>1.04</td>
<td>0.97</td>
</tr>
<tr>
<td>10</td>
<td>Antraquinone</td>
<td>5.37</td>
<td>4.21</td>
<td>3.87</td>
<td>1.22</td>
<td>1.34</td>
</tr>
</tbody>
</table>

Table 1. Phytochemical components of the extract of the leaf of *P. macrocarpus* using different solvents

Table 2. Quantitative phytochemical analysis of *P. macrocarpus* leaf (mg/100g)
Table 3. Effect of *P. macrocarpus* on body weight and blood glucose level in streptozotocin induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Body weight Days of treatment</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>Control</td>
<td>222±3.4</td>
<td>228±5.2</td>
</tr>
<tr>
<td>B</td>
<td>Diabetic control</td>
<td>215±5.6</td>
<td>204±3.5</td>
</tr>
<tr>
<td>C</td>
<td>Glibenclamide (0.5mg/kg)</td>
<td>223±4.5</td>
<td>223±4.5</td>
</tr>
<tr>
<td>D</td>
<td><em>P. macrocarpus</em> 50 mg/kg</td>
<td>222±4.9</td>
<td>226±5.4</td>
</tr>
<tr>
<td>E</td>
<td><em>P. macrocarpus</em> 100 mg/kg</td>
<td>218±3.8</td>
<td>224±2.5</td>
</tr>
</tbody>
</table>

Statistically significant *p*<0.05, compared to diabetic control; Data represented as mean ± SD

Table 4. Changes in Fasting Blood Glucose Level following a 14-day administration of *P. macrocarpus* extract to streptozotocin-diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood glucose (mg/dl)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-STZ treatment</td>
<td>7 days post STZ treatment</td>
<td>14 days post extract treatment</td>
</tr>
<tr>
<td>A</td>
<td>Control</td>
<td>60.52±11.85</td>
<td>68.60±6.73</td>
<td>67.00±11.38</td>
</tr>
<tr>
<td>B</td>
<td>Diabetic control</td>
<td>67.51±8.10</td>
<td>294.34±6.12</td>
<td>438.75±1.16</td>
</tr>
<tr>
<td>C</td>
<td>Glibenclamide (0.5mg/kg)</td>
<td>75.45±7.00</td>
<td>304.12±5.51</td>
<td>201.43±4.13*</td>
</tr>
<tr>
<td>D</td>
<td><em>P. macrocarpus</em> 50 mg/kg</td>
<td>78.67±4.13</td>
<td>270.32±9.15</td>
<td>359.37±4.83</td>
</tr>
<tr>
<td>E</td>
<td><em>P. macrocarpus</em> 100 mg/kg</td>
<td>74.42±7.12</td>
<td>234.65±4.13</td>
<td>140.76±4.83*</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SD, n=5; statistically significant *p*<0.05; compared with the diabetic control group

Fig. 1a and 1b. Photomicrograph of a pancreatic section from the control group showing normal Islet (IL) and Acinar cells (A). Fig. 2a and 2b. Photomicrograph of a pancreatic section from the diabetic control group showing islets atrophy (IL). Fig. 3a and 3b. Photomicrograph of a pancreatic section from the glibenclamide group showing islets cells (IL) and Acinar cells (A). Fig. 4a and 4b. Photomicrograph of a pancreatic section from the *P. macrocarpus* 50 mg/kg group showing islets cells (IL) with vacuole and Acinar cells (A). Fig. 5a and 5b. Photomicrograph of a pancreatic section from the *P. macrocarpus* 100 mg/kg group showing prominent islet cells (IL) and Acinar cells (A). H/E stain

Keys: A- Acinar cells, IL- islet of Langerhans
Fig. 1a, 2a, 3a, 4a and 5a (Mag. X125)
Fig. 1b, 2b, 3b, 4b and 5b (Mag. X500)
3.5 Histological Observations

The histo-architectural integrity of the group A which is the control pancreatic tissue; showing normal islet of Langerhans with cluster of cells and very large blood vessels as seen in (Fig. 1a and 1b). In group B (diabetic control), there was degeneration of (cellular lesion) islets of the pancreas indicated by necrosis, islets atrophy and eosinophilic material (Fig. 2a and 2b). While, group C (0.5 mg/kg glibenclamide) showed a marked increase in size of the islets (Fig. 3a and 3b). Administration of test doses of 50mg/kg of P. macrocarpus, group D showed islets cells, with vacuole and acinar cells (Fig. 4a and 4b). However, group E that received 100mg/kg b.w. of the extract showed prominent, well stained islet and acinar cells (Fig. 5a and 5b).

4. DISCUSSION

Diabetes induced by STZ causes weight loss in animals which is due to lack of insulin and proteinuria. Therefore the lack of insulin stimulate the liver to breakdown protein into amino acids resulting to excessive weight loss [27], which was observed in the study. Among two test doses (50 and 100mg/kg) P. macrocarpus at 100 mg/kg was found to be more effective in increasing the body weight compared to diabetic control rats. This could be due to the protective effect of the extract in controlling muscle wasting.

The majority of islet cells is formed by beta-cells which are responsible for producing insulin. Hence, depletion of beta-cells will therefore lead to lack of insulin which will result in disorder of carbohydrate, protein and fat metabolism and the end result is hyperglycaemia [28]. In this study, induction of diabetes mellitus was done with STZ, which selectively destroy beta-cells of the islet, also deposition of a homogenous eosinophilic material occupying the islet was seen in diabetic untreated, this may be a localized amyloidosis which has been reported to occur in the pancreas in many diabetics [29]. Furthermore, scanty atrophic cells present may be related to chronic inflammatory disorder as a result of the diabetic condition [30]. Islet cells of group E treated with 100mg/kg/day of P. macrocarpus extract has regenerated considerably, suggesting the presence of stable cells in the islets that have the ability of regenerating [31]. This also suggests that the P. macrocarpus leaf extract at this dose has the ability of inducing the quiescent cells to proliferate to replace the lost cells.

Preliminary phytochemical screening of the plant extract of P. macrocarpus showed the presence of alkaloid, saponin, tannin, phenol, flavonoid, cardiac glycoside, steroids, terpenoids, anthocyanin and anthraquinone. It have been reported by a number of investigators that tannins, saponins, flavonoids and other secondary metabolites from plant possess hypoglycemic and other pharmacological activities [32,33]. Some researchers have reported that the flavonoids present in plant have potent antidiabetic activities by decreasing blood glucose, increasing the number of beta-cells and recovery in the altered biochemical variables [34,35]. Furthermore, phenolic content of plants also contributes to antioxidant activity of plants materials by mopping up the reactive oxygen species produced by the STZ to damage the beta-cells [36]. Tannins possess anti-inflammatory and antioxidant activities [37], anthocyanins have been proven to have antioxidant properties [38]. Moreover, terpenoids medicinal properties includes antihyperglycemic and anti-inflammatory properties [39,40]. Therefore, the anti-oxidant activities of the extract may be connected with the antidiabetic activities of the plant. Also, antioxidants from other medicinal plants have also been reported in the management of diabetes [41,42,43]. It is obvious that P. macrocarpus plant extract is able to reduce hyperglycemia by increasing glucose metabolism and also cause regeneration of pancreatic beta-cells at a dose 100 mg/kg/day either by its active anti-oxidants components acting together or single. The study on antidiabetic activity of the ethanolic extracts of P. macrocarpus leaf showed significant anti-hyperglycemic activities in STZ-induced diabetes.

5. CONCLUSION

In conclusion, the ethanolic leaf extract of Petersianthus macrocarpus has an anti-hyperglycemic activity on the pancreas at a dose of 100 mg/kg body weight in STZ-induced diabetic rats. This justifies its use in folklore medicine in managing diabetes. However, there is need to identify the phyto-components that may be responsible for the antihyperglycaemic activity.

CONSENT

This is not applicable.

ETHICAL APPROVAL

The Experiment was approved by Research Ethics Committee of Faculty of Basic Medical
ACKNOWLEDGEMENTS

The authors are grateful to the following for their technical assistance; Mr. Justice Okoronkwo of National Root crops Research Institute, Umudike- Umuahia and Mr. Agbakwuru of the Department of Anatomy, College of Veterinary Medicine, Federal University of Agriculture, Umudike Abia State.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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